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FREEZING CURVE BASED MONITORING TO QUICKLY EVALUATE THE VIABILITY OF THE BIOLOGICAL MATERIALS SUBJECT TO FREEZING AND REWARMING

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Abstract-**A new technique based on freezing curve monitoring to quickly evaluate the viability of the biological materials subject to freezing or rewarming was established. A practical integrated device was fabricated which is simple in structure and cheap in price. Preliminary freezing experiments on the fresh fish blood demonstrated that minor changes in a biological material due to freezing or warming injury may result in significant deviation in its freezing curve compared with that of the intact biomaterials. Three thermal indexes to quantify the damage degree of the biomaterials were pointed out. This method also opens many opportunities for the evaluation of biological material defect in diverse life science fields.**

***Keywords*-Cryo-biology, cryosurgery, biomaterials, viability evaluation, freezing property, biological chip, electric cooling device**

I. INTRODUCTION

There are increasing public interests on the low temperature biology and medicine [1]-[4]. For example, cryosurgery is being extensively used for the controlled destruction of non-desirable tissues in dermatology, proctology, gynaecology, neurosurgery and veterinary medicine. And cryopreservation is also widespread in the preservation of sperm, blood, yeast cells, hepatocytes, skin, cornea, bone, tissues, vessel, fungi, human heart valves and other organs. In all these practices, freezing was used either to preserve or to kill the biomaterials. Thawing is the integral part of a complete cryosurgery or cryopreservation process. At those widely different cooling and warming rates maximal preservation or destruction of biological materials is achievable [5], [6]. In order to obtain an optimal output, specific cooling and warming rates must be imposed during the cryo-medical process. Assessing the freezing and warming damage of the biological materials and correlating the final result to a specific program are thus critical for the practices [4]. Here we show a highly quantitative method aiming at accelerating the process to find optimum program among various freezing and warming conditions. It may also possibly be extended to test a wide variety of biological materials related to cell destruction.

II. METHODOLOGY

Due to diversity in structure, composition, concentration and storage conditions for different biomaterials, no universal methods are available to assess their viability after cryo-preservation or surgery. The most typical ways include: morphological and physiological observation, cell culture followed with biochemical testing, fluorescence detection, long-term histological evaluation, cell accounting by flow cytometric analysis, liquid chromatography, composition analysis, isotope marker method, electron spin resonance spectroscopy and nuclear magnetic resonance technique, oxygen or glucose consumption analysis, dye exclusion and dopamine release, dielectric measurement, differential scanning calorimetry, effective thermal conductivity measurement, minimum cell-to-volume ratio. Most of these methods appear either too complicated or request tedious preparation. And they are easily affected by the external factors, usually time consuming and expensive. Therefore, establishing a quick and cheap method to find out the best freezing or warming program has been an important issue. As has been recognized, freezing or thawing affects biological systems at both nanoscale (molecular) and microscale (cellular) levels [7]. And the site most easily damaged is the cell membrane that surrounds the intracellular solution [7]. The phospholipid bilayers that form the cell membrane undergo an abrupt phase change from a disordered fluid to solid when subject to an external freezing. This phase change temperature is strongly dependent on the composition of the phospholipids, their chain length and degree of saturation [7]. Since non-desirable freezing or warming will irreversibly change the cell units consisting of the biological systems, it usually results in a shifted freezing temperature behavior for the processed sample, compared with that of the intact material. It is from this shift can the damage degree of the cryopreserved and thawed biomaterials be quickly assessed. This becomes the foundation of our current new method.

III. DEVICE FABRICATION

To demonstrate this strategy, an integrated device with five layers was constructed as depicted in Fig.1. The first 3mm thick layer is made of plexiglass and used as the cover of the sample container to avoid external disturbances. The second 3mm thick layer is also made of plexiglass drilled with one cylindrical hole (serving as sample container) whose diameter is about 5mm. Immediately below this layer is the third one made of 0.5mm thick copper film, which also serves as the bottom of the mini-container. The fourth 5mm thick layer is the thermoelectric cooling unit (TECU), which is made of commercially available Peltier elements. Grease with high thermal conductivity was sandwiched between copper film and the fourth layer to reduce their thermal contact resistance. Immediately below the bottom of the TECU is the fin-structured layer made of aluminum which was used to enhance dissipation of heat generated in the TECU and to improve cooling performance. To keep the

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Abstract

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TECU work safely and steadily, the fin structure was immersed in a water bath with fixed temperature of 20°C. Layers from second to the fifth were tightly bolted together. The carefully calibrated T-type thermal couples with filament diameters of 100μm and data acquisition system (USA, Agilent 34970A) were applied to monitor the temperature of the sample. The thermal couples were mounted on the bottom of the mini-container and exposed to the sample to obtain an accurate measurement.

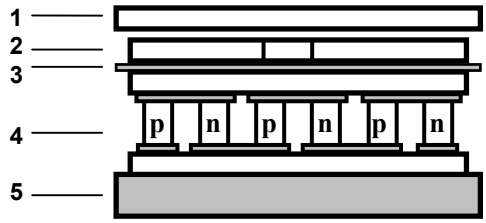

Fig.1. Schematic of the device to apply cooling and record temperatures. 1-the plexiglass cover; 2--the plexiglass layer symmetrically drilled with mini-container; 3--the copper film layer; 4--the thermoelectric layer for cooling/heating; 5--fin structure to enhance heat dissipation.

IV. RESULTS AND DISCUSSION

A preliminary qualitative experiment was performed on the fresh fish blood. Drain 50 mg of fish blood and place it into the mini-container. Cool the sample with the TECU designed in Fig.1 and monitor its freezing curve using the data acquisition system. The recorded temperature transient for this blood was displayed as the solid curve in Fig.2. After that, turn off the TECU and leave the sample and the container in the air to slowly warm up until it reaches the room temperature and keeps unchangeable in temperature. Then a complete freezing-thawing cycle for the blood was finished. At this time, turn on the TECU with the same power as before and the blood temperature will decrease again due to freezing. The temperature transient in this case was recorded and depicted as the dashed curve in Fig.2. Comparing the two curves in Fig.2, one can find quite a few interesting results. Although the two temperature decreasing curves appear the same at the early stage of cooling, they will gradually deviate from each other. Especially at the time when the phase change occurs, this deviation becomes most evident. Fig.2 indicates that, there appears a temperature jump in each curve. This is due to heat release from commences of the phase change in the sample. Under the continuous cooling, the temperature will drop again after passing the phase change point, resulting in a plateau over the freezing curve. The difference between the two plateaus is so large that it can serve as one of the best indexes to quantify the sample property. The time to initiate the temperature jump and the time to end it deviate significantly between the two curves. For the blood sample which had experienced one freezing-thawing cycle, the time to initiate the temperature jump during the second freezing is at about 64s (see dashed curve in Fig.2). While for the fresh blood subject to the first freezing, it is at about 84s (see solid curve in Fig.2). Over the whole time interval, the highest temperature difference at the same time for the two curves can even be as high as 15ºC, which is detectable by any temperature sensors. Clearly, due to the freezing by TECU and slow warming by air, the micro-scale structure of the fish blood with red cells has been damaged in some degree. Although one can not distinguish the change in the sample appearance after its recovery to the normal temperature, the significant deviation in the freezing curve for different situation reveal the interior property of the sample. It is in this way can the viability of the pre-frozen and thawed biomaterial be quickly assessed. For this purpose, several indexes can be used to quantify the degree of the property deviation between the pre-frozen and the intact samples. One is the time for the temperature to jump in the freezing curves. Another is the maximum temperature difference between the two curves. The third index can be the sum of the absolute area difference between the two curves.

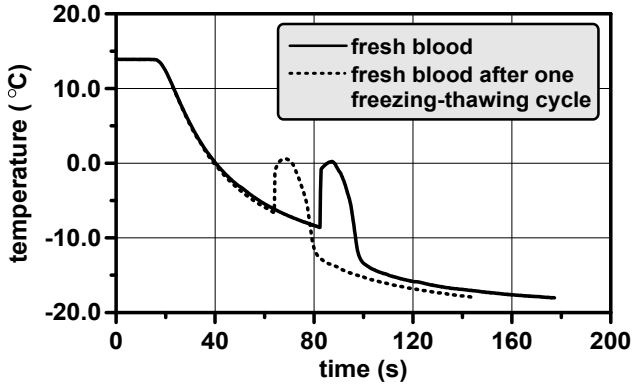

Fig.2. Time dependent freezing curves for the fresh blood and the pre-frozen and air slowly thawed blood sample. The blood was taken from the abdomen of the crucian fish just sacrificed. Both curves are subject to the same cooling by TECU.

V. CONCLUSION

Previously, freezing or the subsequent warming process is usually left to an operator to judge, which turns out to be unreliable. Study in this paper demonstrates the possibility to instantly evaluate the viability of the biomaterials through monitoring their transient freezing curves. Compared with most of the currently existing methods, the present approach is extremely simple and highly quantitative.

Clearly, applications of the present method are not limited to the cryo-medical field. Among potential applications is assessment of a hyperthermia process using a variety of heating apparatus such as microwave, laser, and ultrasound to damage the tumor cell. And quickly finding the optimum procedure among various heat deposition patterns will benefit from this freezing monitoring method. If the biomaterial was destroyed by the high temperature, it is expected to have a shifted freezing curve compared with that of the intact material. The study also implies that, using temperature index to detect other material properties such as biological structure, composite, mechanical property, food

quality, degradation kinetics and biocompatibility even molecular features is also worth of investigation. And the tested materials will not be confined to the biological samples. Therefore, it is in great need to expand applications of the present method.

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